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Leucoagaricus pabbiensis sp. nov. from Punjab, Pakistan

Muhammad Usman* & Abdul Nasir Khalid

Fungal Biology and Systematics Research Laboratory, Department of Botany, University of the Punjab, Quaid-e-Azam Campus-54590, Lahore, Pakistan

* Correspondence to: *musmanmughal52@yahoo.com*

Abstract—A novel species, *Leucoagaricus pabbiensis*, is described and illustrated from Pabbi Forest Park, Punjab, Pakistan. The new species belongs to *Leucoagaricus* sect. *Leucoagaricus* and is characterized by a dark reddish brown central disc on a milky white pileus with radial reddish orange fibrils towards the margin. The creamy stipe, which is longer than the pileus is wide, has an ascending annulus; the basidiospores are lacrymoid to amygdaliform and dextrinoid; cheilocystidia are clavate to narrowly clavate; and both stipitipellis and pileipellis are made up of septate hyphae arranged as a cutis. The new agaric closely resembles *L. badius* but the novelty of *L. pabbiensis* is confirmed by morphological and molecular characters.

Key words—*Agaricaceae*, Gujrat, ITS region, macrofungi, Pakistan

Introduction

Salient characters of the genus *Leucoagaricus* Locq. ex Singer (*Agaricaceae*) are a fibrillose to squamulose pileus surface, white to cream or pink spore print, non-striate pileus margin, free lamellae, dextrinoid spores that are metachromatic in cresyl blue, and hyphae lacking clamp connections (Singer 1986, Vellinga 2001). *Leucoagaricus* is one of the basidiomycete genera with an extensive geographical distribution. More than 100 species are distributed from temperate to tropical regions of the world. Recent reports of new *Leucoagaricus* species throughout both northern and southern hemispheres suggest that many species of *Leucoagaricus* are still undescribed (Kumar & Manimohan 2009, Vellinga 2010, Vellinga & al. 2010, Liang & al. 2010, Vellinga & Balsley 2010,

Ge 2010, Muñoz & al. 2012, 2014, Malysheva & al. 2013, Kumari & Atri 2013, Ye & al. 2014, Ge & al. 2015, Qasim & al. 2015, Dovana & al. 2017, Hussain & al. 2018).

Leucoagaricus is poorly studied in Pakistan, and so far only nine species have been reported: three from Khyber Pakhtunkhwa province—*L. badius* S. Hussain & al, *L. pakistanensis* Jabeen & Khalid, *L. sultanii* S. Hussain & al.—and six species from lower elevations in district Lahore, Punjab province—*L. asiaticus* Qasim & al., *L. lahorensis* Qasim & al., *L. lahorensiformis* S. Hussain & al., *L. leucothites* (Vittad.) Wasser, *L. serenus* (Fr.) Bon & Boiffard, and *L. umbonatus* S. Hussain & al. (Ahmad & al. 1997, Qasim & al. 2015, Ge & al. 2015, Hussain & al. 2018).

Pabbi Forest Park (32°49′53″N 73°50′16″E) is part of the Himalayan foothills located in Gujrat district (Khan & al. 2016). The entire park covers about 160 km^2 over altitudes ranging from 280 to 410 m. Its rangeland vegetation type includes *Acacia modesta*, *Grewia tenax*, and *Prosopis juliflora* in the upper storey accompanied by the grasses *Cenchrus ciliaris*, *Cymbopogon jwarancusa*, *Cynodon dactylon*, and *Heteropogon contortus* (Arshadullah & al. 2007). Mean annual rain fall is 774 mm, concentrated mainly in July and August, and daily temperatures average 12°C in January and 33°C in June (Haider & al. 2011). Pabbi Forest Park was visited many times during the 2016–17 rainy season as part of a wider survey of the macrofungi of Pakistan. During these visits, two unknown *Leucoagaricus* specimens were collected. Preliminary morphological analysis indicating that the two specimens are conspecific was confirmed by ITS sequence analyses; they are proposed here as a new species, *L. pabbiensis*.

Material & methods

Morphology

Fresh basidiomata were described macroscopically and photographed with a Nikon D70 camera fitted with a Nikkor macro 18–77 mm lens. The specimens were dried under fan heater. Voucher specimens were deposited in the Herbarium, Department of Botany, University of the Punjab, Quaid-e-Azam Campus, Lahore, Punjab, Pakistan (LAH). Description terminology follows Vellinga (2001) and colors were coded according to Munsell (1994). The micromorphological descriptions are based on dried material rehydrated in 2% KOH. Melzer's reagent was used for amyloidity, and Congo red was used for staining cell walls of basidiomatal elements. Observations and measurements were recorded with the help of trinocular Labomed CXRII microscope at 4×, 10×, 40×, and 100× (oil immersion). The abbreviation [n/m/p] indicates n basidiospores measured from m fruit bodies of p collections. At least 20 basidiospores plus 10 each of basidia, cystidia, and pileipellis and stipitipellis elements were measured from one

Fig. 1. *Leucoagaricus pabbiensis* (holotype, LAH35302): basidiomata.

specimen. Basidiospore dimensions were recorded as (a–)b–c(–d), where a = extreme minimum value, range b–c contains at least of 90% of the calculated values, and d = extreme maximum value; Q indicates individual spore l/w ratios and Q_{av} presents the average Q of all spores. Plant names were retrieved from the International Plant Name Index (http://www.ipni.org) and fungal names were retrieved from Index Fungorum (http://www.indexfungorum.org).

DNA extraction, PCR amplification, & DNA sequencing

DNA was extracted following the CTAB method (Bruns 1995). Universal primers ITS1 and ITS4 were used to amplify the rDNA Internal Transcribed Spacer region (ITS). PCR conditions followed Gardes & Bruns (1993). Forward and reverse sequences were obtained from BGI (China) also using the ITS1/ITS4 primer pairs, with a consensus alignment of those sequences reached using BioEdit ver. 7.2.5 (Hall 1999). Nucleotide sequences were compared by using BLAST at NCBI (https://www.ncbi.nlm.nih.gov/guide/). Multiple sequences were aligned online using MUSCLE (https://www.ebi.ac.uk/Tools/msa/muscle/). Sequences with >83% identity (and E = 0) and recently deposited sequences from species of *Leucoagaricus* sect. *Leucoagaricus* (formerly *L.* sect. *Rubrotincti;* Redhead 2016) from Pakistan and other parts of Asia were included in the final data set to reconstruct the phylogeny. The phylogram, generated in RAxML-HPC2 using XSEDE tool (8.2.10), presents 39. sequences of *Leucoagaricus* plus one sequence of *Cystolepiota seminuda* (Lasch) Bon as outgroup.

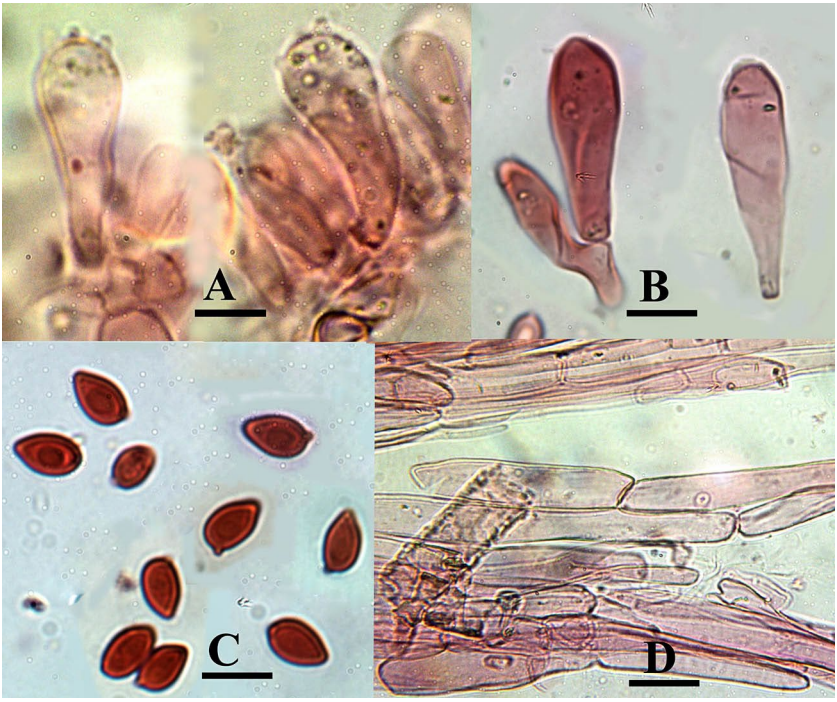

Fig. 2. *Leucoagaricus pabbiensis* (holotype, LAH35302): A. basidia; B. cheilocystidia; C. basidiospores; D. stipitipellis. (All mounts prepared in Congo red.) Scale bars: A = 10 µm, B = 9.5 µm, C = 8.3 µm, D = 7.2 µm.

Taxonomy

Leucoagaricus pabbiensis Usman & Khalid, **sp. nov.** Figs 1–3

MycoBank 824295

Differs from *Leucoagaricus badius* by its milky white pileus with dark reddish brown central disk and radially arranged reddish to orange fibrils, and its larger basidia and basidiospores.

Type: Pakistan. Punjab province, Pabbi Forest Park, 286 m a.s.l, on soil and leaf litter under *Acacia modesta* Wall., 11 August 2016, Muhammad Usman & Abdul Nasir Khalid MU02 (**holotype,** LAH35302: GenBank MG973423).

Etymology: The specific epithet "*pabbiensis*" (Latin) refers to the name of Pabbi Forest Park, the type locality.

Pileus 25–50 mm, when young convex, expanding to plane when mature, milky white with dark reddish brown (7.5R3/6) central disk and radially

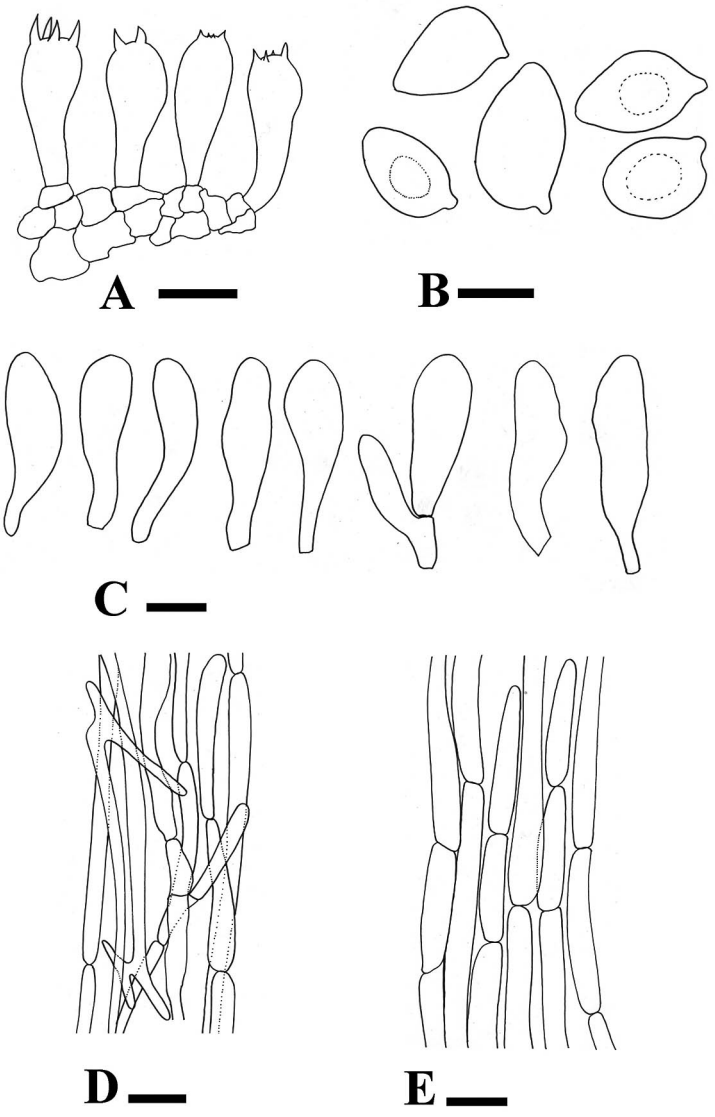

Fig. 3. *Leucoagaricus pabbiensis* (holotype, LAH35302): A. basidia; B. basidiospores; C. cheilocystidia; D. pileipellis; E. stipitipellis. Scale bar A = 16.5 µm, B = 4.5 µm, C = 10 µm, D = 10 µm, E= 12 µm. (Drawings by Muhammad Usman.)

arranged orangish yellow fibrils, orange brown (7.5R5/10) to light orange (7.5R5/16) scales radially arranged, fibrils uplifted; margins eroded. Lamellae free, milky white, crowded, thick, with eroded edge; short lamellulae present in series of 1–3. Stipe 25–60 × 3–5 mm, cylindrical, with subbulbous base, ≤10 mm in diameter, creamy white, smooth, sometimes with some very light pink brownish fibrils, hollow, with basal mycelial pad. Annulus thick, ascending, superior, persistent, creamy white; volva absent. Taste and aroma not recorded.

Basidiospores [50/3/2] (6.7–)7.7–9.6(–10.6) × (4.2–)4.7–5.5(–5.9) µm, on average 8.7 × 5.1 µm, Q = (1.32–)1.5–1.9(–2.2), Q_{av} = 1.7, hyaline in 2% KOH, dextrinoid, lacrymoid and amygdaliform in side-view, guttulate, smooth, thin-walled, apiculate. Basidia 20–32.4 × 8.5–11.4 µm, clavate, 2–4-spored, smooth with 2–4 prominent sterigmata. Cheilocystidia 28.3–36.2 × 8.6–12.3 µm, hyaline in 2% KOH, thin-walled, clavate to narrowly clavate, smooth. Pleurocystidia absent. Pileipellis a cutis, made up of 3.1–9.1 µm wide, septate, thin-walled hyphae, hyaline in 2% KOH; clamp connections absent. Stipitipellis made up of septate hyphae 4.8–8.9 µm in diameter, hyaline in 2% KOH, thin-walled, parallel in arrangement. Clamp connections absent.

Ecology & distribution—Solitary to gregarious on moist soil, rich in leaf litter under trees of *Acacia modesta*, fruiting during the monsoon season.

Additional specimen examined—**PAKISTAN**. **Punjab province**, Pabbi Forest Park, 286 m a.s.l, 20 August 2017, Muhammad Usman & Abdul Hameed MU78 (LAH35303: GenBank MG973424).

Phylogenetic analysis Fig. 4

The phylogenetic tree comprises 40 sequences, of which 39 represent the ingroup, *L.* sect. *Leucoagaricus*. The final data set comprised 713 characters, of which 425 were conserved, 279 were variable, 188 were parsimony informative, and 91 were singletons.

The two *L. pabbiensis* sequences MG973423 and MG973424 were 100% identical, indicating that they are conspecific. In BLAST, the *L. pabbiensis* sequences showed maximum identity to a sequence named *Leucoagaricus* sp. from India (KR154966); and in the phylogenetic tree, these three sequences formed a clade with 94% bootstrap support (Fig. 4). *Leucoagaricus pabbiensis* also showed 99% similarity in BLAST with *L. badius* from Pakistan (KU647734–KU647736), but our phylogenetic analysis placed these two species in sister clades. *Leucoagaricus pakistanensis* (KU647726–KU647728), which is 91% identical to *L. pabbiensis,* forms a separate clade. Other *Leucoagaricus* species reported from Pakistan are *L. lahorensis* (KJ701794, KJ701796) 87% identical

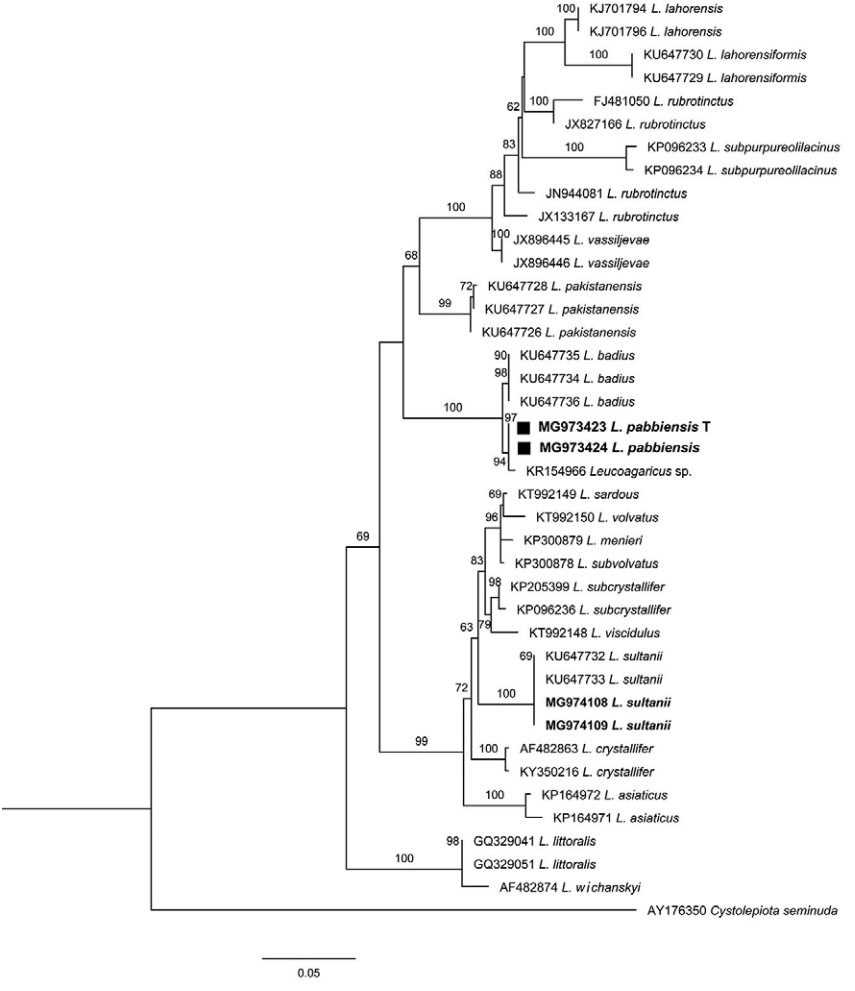

Fig. 4. Phylogram obtained from ITS sequence alignment of species of *Leucoagaricus* sect. *Leucoagaricus*. Bootstrap values >67% shown at nodes. *Leucoagaricus pabbiensis* sequences are indicated by ▪; and the type sequence is annotated **T**.

with *L. pabbiensis; L. lahorensiformis* (KU647729, KU647730) 87% identical; and *L. sultanii* (MG974108, MG974109, KU647732, KU647733) 90% identical. *Leucoagaricus rubrotinctus* (Peck) Singer (JN944081) from North America is 89% identical, and *L. subpurpureolilacinus* Z.W. Ge & Zhu L. Yang (KP096233, KP096234) from China is 87% identical.

Discussion

Leucoagaricus pabbiensis is characterized morphologically by a milky white pileus with dark reddish brown central disk and reddish to orange fibrils radiating towards the margin, lacrymoid to amygdaliform basidiospores, a cylindrical stipe with light pink fibrils and an ascending annulus. This set of morphological characters and phylogenetic analysis distinguishes *L. pabbiensis* from other closely related species in *L.* sect. *Leucoagaricus* from Pakistan and other parts of the world.

Leucoagaricus badius, a similar species from Malakand, Khyber Pakhtunkhwa, Pakistan, differs from *L. pabbiensis* by its white pileus with medium to dark red fibrillose squamules and deep red umbo, and its smaller basidiospores (6.5–7.5 × 4–5 µm) and basidia (14–17 × 6–8 µm; Hussain & al. 2018).

Another closely related species, *L. pakistanensis,* differs from *L. pabbiensis* by the color of its pileus and its smaller cheilocystidia (15–20 µm long; Hussain & al. 2018). *Leucoagaricus lahorensis* morphologically resembles *L. pabbiensis* only in the reddish color of its pileus and differs by its ellipsoid to broadly ellipsoid, larger basidiospores (8–10.6 × 6.4–7.6 µm; Qasim & al. 2015); our phylogenetic analysis (Fig. 4) also supports *L. pabbiensis* as distinct from *L. lahorensis. Leucoagaricus lahorensiformis* also shares some similarities with *L. pabbiensis* but differs by its smaller pileus (10–30 mm), basidiospores (6.5–7.5 × 3.5–4 µm), and basidia (18–22 × 6–9 µm) (Hussain & al. 2018).

Leucoagaricus pabbiensis also resembles *L. rubrotinctus,* the type species of the genus (Redhead 2016), first described from North America; but in *L. rubrotinctus* the whole pileus is orange-red and covered by orange fibrils, without showing the white context (Peck 1884, as *Agaricus rubrotinctus* Peck, nom illeg.). Phylogenetically these two species form separate lineages.

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